



## Pilot study of temporal variations in lead bioaccessibility and chemical fractionation in some Chinese soils

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### ABSTRACT

The effect of ageing, following the addition of  $\sim 400 \text{ mg kg}^{-1}$  lead (Pb) as  $\text{Pb}(\text{NO}_3)_2$ , on Pb bioaccessibility was examined in five typical Chinese soils using a physiologically based extraction test. Sequential extraction was employed to identify the source fraction(s) of bioaccessible Pb in the soils. Pb bioaccessibility decreased exponentially to nearly steady levels in mildly acidic or alkali (pH 6.09–7.43) soils, for both gastric (69.91–71.75%) and small intestinal (7.53–9.63%) phases within the first 2–4 weeks and 1–2 months of incubation, respectively; however, it took only 1–2 weeks for strongly acidic ( $\approx$  pH 4.5) soils to reach nearly steady levels of Pb bioaccessibility (73.01–74.46% and 10.30–10.98% in the gastric and small intestinal phases, respectively). In addition to the water-soluble and exchangeable fractions, the carbonate fraction of mildly acidic or alkali soils appeared to be a third main source of bioaccessible Pb in the small intestinal phase; however, bioaccessible Pb was likely to derive principally from Pb in the water-soluble and exchangeable fractions of strongly acidic soils. Bioaccessible Pb in the gastric phase appeared to derive from all the fractions in all five studied soils, even the residual fraction.

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### 1. Introduction

Lead (Pb) has been recognized as a contaminant posing threats to both soil quality and human health. Two main sources of Pb contamination of soils are industrial activities, including mining and smelting of nonferrous metals, and historical use of Pb-containing products such as paint, leaded gasoline and pesticides [1].

Exposure to Pb levels sufficient to produce blood Pb concentrations of  $10\text{--}15 \mu\text{g dL}^{-1}$  has been linked to undesirable developmental outcomes in human fetuses and children [2]. Canfield et al. [3] reported that even below  $10 \mu\text{g dL}^{-1}$ , blood Pb levels are inversely correlated to children's IQ scores at 3 and 5 years of age; in fact, associated declines in IQ were greater at these levels than at higher levels, indicating that more children may be adversely affected by environmental Pb than previously estimated. Researchers with the Chinese Medical Association found that 65% of 11,348 school children tested had blood Pb levels above

the safe limit of  $10 \mu\text{g dL}^{-1}$  set by the World Health Organization [4].

Soils often act as a sink for heavy metals derived from various anthropogenic sources. Therefore, soil contamination with heavy metals has attracted interest, due to both environmental and health-risk concerns [5,6]. Inadvertent oral ingestion of soil is considered an important exposure pathway for metals and other contaminants, especially in children through outdoor hand-to-mouth activities [7]. Previous work has reported a range of values for bioavailable contaminant levels in soils, with most values being less than 100%, and the age and physical condition of the soil had a significant impact on contaminant bioavailability in each study [8,9]. In the last few decades, the methodology for determining the bioavailability of soil heavy metals to humans has seen rapid development, although no standard method of estimating bioavailability has yet been approved by regulatory agencies. Physiologically based extraction (*in vitro*) tests, which have been well-validated through comparison with animal (*in vivo*) tests, have proven to be a fast, cost-effective and reliable approach for estimating the bioavailability of heavy metals in soils to humans [10–13]. *In vitro* methods have been used to estimate the bioaccessibility of heavy metals (e.g. Pb, cadmium and chromium) in soils near ore smelters, mine tailings and other polluted sites [10–17], as well as to evaluate the effectiveness of soil remediation technologies [18–21].

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Plant uptake and toxicity of metals can be related to specific fractions of a sequential extraction procedure using progressively harsher reagents to dissolve increasingly refractory forms [22], although some inherent limitations caused by non-selectivity of the reagents and possible re-adsorption of metals during the extraction have been reported [23]. Sequential extraction procedures have been widely employed not only to examine the physicochemical forms of metals and thus enable a better understanding of the processes influencing their availability [24], but also to assess the effectiveness of soil remediation technologies and reveal underlying mechanisms [25,26]. A comparative study of Pb bioaccessibility in 15 soils from Germany with Pb pools from a sequential extraction procedure showed that the addition of milk could increase Pb bioaccessibility by depleting the organic Pb pool [17]. Sequential extraction procedures have also been used to investigate temporal changes in added heavy metals in soils [27–29]. The decreasing bioavailability of a metal to a given biological organism as its contact time with the soil increases is often termed “ageing” through specific retention mechanisms which are still not well understood. A number of mechanisms may be operational in trace element retention, and the dominant mechanism may vary with incubation time, as described elsewhere [27–28]. Under worst-case bioavailability scenarios, such as new or ongoing site contamination, there is a clear need to intensively investigate the temporal variations in the health-risk assessment, which could result from temporal variations in the physicochemical forms of heavy metals such as Pb.

Nevertheless, to the best of our knowledge, few investigations in the literature have focused on revealing the effect of the ageing process and its underlying mechanisms on the bioaccessibility of heavy metals such as Pb in soil. Fendorf et al. [28] reported that Pb bioaccessibility in the B horizon of soil from the Oak Ridge National Laboratory site declines rapidly to less than 20% within 30 days of soil incubation with a mixture of NaAsO<sub>2</sub>, CrCl<sub>3</sub> and PbCl<sub>2</sub> in a 0.001-M CaCl<sub>2</sub> matrix at pH 3 in a 1:10 (w:v) soil-to-solution ratio, whereas exchangeable Pb extracted by MgSO<sub>4</sub> is relatively constant with time; however, this study was conducted only in the gastric phase, without further evaluation in the small intestinal phase where the main adsorption process usually occurs [30].

The current study was aimed at revealing temporal variations in Pb bioaccessibility in some typical soils of China using both a modified physiologically based extraction test (PBET) and a sequential extraction procedure, in an attempt to identify the source fraction(s) of bioaccessible Pb in soils and the effects of soil properties. Such investigations could be crucial in assessing temporal variations in health risks at either new or historically Pb-contaminated sites.

## 2. Materials and methods

### 2.1. Soil preparation

Five typical uncontaminated soils, including Cryi-Ustic Isohumosol (CC), Hapli-Ustic Argosol (BJ), Ferri-Udic Argosol (NJ), Haplic-Udic Ferrosol (YJ) and Rhodi-Udic Ferrallosol (HN), were collected from uncultivated lands located across China, from north to south. Selected chemical and physical soil properties, including the background Pb concentration, are shown in Table 1. Soils were air-dried, ground and passed through a 2-mm mesh sieve. The chemical form of Pb-containing contaminants in soils is usually highly variable and dependent on the nature of their industrial source. Therefore, the soluble Pb salt Pb(NO<sub>3</sub>)<sub>2</sub> was chosen in this study for addition to the soils in order to reveal Pb-partitioning patterns and their potential relation to temporal variations in bioaccessible Pb levels following the addition of free Pb ion. Use of this

**Table 1**  
Selected physical and chemical properties of soils

Soil	CC	BJ	NJ	YJ	HN
pH (CaCl <sub>2</sub> )	6.09	7.43	6.09	4.50	4.56
CaCO <sub>3</sub> (g kg <sup>-1</sup> )	8.75	36.88	7.88	4.38	2.38
Organic carbon (g kg <sup>-1</sup> )	15.40	41.02	26.46	3.68	4.86
Available P (mg kg <sup>-1</sup> )	13.34	15.02	12.80	2.02	1.14
CEC (cmol kg <sup>-1</sup> )	22.50	15.75	23.25	10.88	6.75
Fe (g kg <sup>-1</sup> ) <sup>a</sup>	4.22	4.04	6.51	22.56	48.85
Mn (g kg <sup>-1</sup> ) <sup>a</sup>	0.27	0.15	0.46	0.09	0.51
Pb (mg kg <sup>-1</sup> )	12.80	29.53	36.15	20.71	21.01
Particle size composition (%)					
Sand (>0.05 mm)	5.1	5.1	4.0	0.9	3.0
Silt (0.002–0.05 mm)	82.2	77.3	88.1	58.8	64.3
Clay (<0.002 mm)	12.7	17.6	7.9	40.3	32.7
Clay minerals <sup>b</sup>	i > s	i > v > s	i > s > k	k > v > i	k > h > g

<sup>a</sup> Represents free Fe/Mn-oxide.

<sup>b</sup> g, gibbsite; h, hematite; i, illite; k, kaolinite; s, smectite; v, vermiculite.

salt allowed us to avoid involvement of the kinetic dissolution process of some sparsely soluble Pb-containing minerals or chemicals (e.g. cerussite) that may be found in various contamination scenarios. Samples (400 g) of each soil were artificially spiked with 80 mL of Pb(NO<sub>3</sub>)<sub>2</sub> solution (2000 mg Pb L<sup>-1</sup>) to increase the total soil Pb concentration by about 400 mg kg<sup>-1</sup>, and then mixed thoroughly. After air-drying and re-homogenizing by 2-mm sieving, 40 g of sub-sample was weighed into a plastic cup and kept in the dark at 25 °C. To simulate the wetting–drying cycles in the field, soil moisture was brought back to 15% from 7 to 8% by adding double-distilled water on a weekly basis. Soil samples were air-dried 1, 3, 7, 14, 30, 60 and 120 days after the artificial Pb addition, and then ground gently with a wooden stick. Since it is usually assumed that only soil particles less than 0.25 mm in diameter adhere to the hands of children [31], only this soil fraction (<0.25 mm) was used for both the physiologically based extraction test and the sequential extraction.

### 2.2. Soil characterization

Soil pH was determined in 0.01 M CaCl<sub>2</sub> with a pH meter in a 1:2.5 (w:v) soil-to-suspension ratio after 1 h of equilibration. Soil contents of carbonate and organic carbon were measured using the acid-neutralization [32] and wet-oxidation [33] methods, respectively. Available P was determined by extraction with 0.5 M NaHCO<sub>3</sub> (adjusted to pH 8.5) for 30 min in a 1:20 soil-to-solution ratio [34]. Cation-exchange capacity (CEC) of the soil was measured by NH<sub>4</sub><sup>+</sup> retention after percolation with 1 M NH<sub>4</sub>OAc solution (pH 7.0) [35]. Free Fe and Mn oxides were extracted with bicarbonate-citrate-dithionite [36], and their levels determined by inductively coupled plasma-atomic emission spectrometry (ICP-AES) or inductively coupled plasma-mass spectrometry (ICP-MS). The background concentration of Pb in the soil was analyzed by ICP-MS after digesting uncontaminated soil samples (<0.25 mm) with aqua regia (a mixture of concentrated HNO<sub>3</sub> and concentrated HCl in a volumetric ratio of 1:3) and perchloric acid at 160 °C.

After dispersing the soil with 0.5 M NaOH for strongly acidic soils YJ and HN, and with 0.5 M sodium oxalate for higher pH soils CC, BJ and NJ [37], particle-size composition of the <0.25-mm size fraction was determined by sieving and weighing for the 0.02–0.25-mm fractions and with a laser particle size analyzer (Mastersizer 2000, Malvern Co., Worcestershire, UK) for the <0.02-mm fraction. Soil clay mineralogy was characterized by X-ray diffraction (XRD) analysis using Cu K $\alpha$  radiation. Mg- and K-saturated samples were scanned at 2 $\theta$  min<sup>-1</sup> on ceramic tiles at 25 °C. The K-saturated tiles were further scanned following heat treatments of 110, 300, and 550 °C [38].

### 2.3. Physiologically based extraction test

The physiologically based extraction test proposed by Ruby et al. [10] was used in this study with a major modification involving the addition of 0.15 M NaCl, as proposed by Rodriguez et al. [7]. Artificial gastric solution was prepared by adjusting 4 L of deionized water to pH 1.5 with 12 M HCl and then adding 35.1 g NaCl, 2 g citrate, 2 g malate, 1.68 mL lactic acid, 2 mL acetic acid and 5 g pepsin (P7000, Sigma Chemical Co., St. Louis, MO, USA) into the solution. Soil (6 g) was added to 600 mL of artificial gastric solution in a 1-L glass reaction vessel which was approximately four-fifths submerged in a temperature-controlled (37 °C) water bath. The anaerobic condition of the digestive tract was created by constantly diffusing argon gas at 1 L min<sup>-1</sup> through the solution, and solution pH was monitored constantly and adjusted to the selected pH with concentrated HCl or NaHCO<sub>3</sub> powder (rather than saturated NaHCO<sub>3</sub> solution to avoid a significant change in solution volume), as necessary, throughout the procedure. After 1 h in the gastric phase, the artificial gastric solution was modified to the small intestinal solution by adjusting the pH from 1.5 to 7 with NaHCO<sub>3</sub> powder and adding 1.2 g porcine bile extract (B8631, Sigma Chemical Co., St. Louis, MO, USA) and 0.36 g porcine pancreatin (P1500, Sigma Chemical Co., St. Louis, MO, USA) to each reaction vessel. The duration of the small intestinal phase was 4 h.

Constant mixing was performed throughout the procedure using paddle stirrers at a speed of approximately 100 rpm. After each phase, a 20-mL sample of the suspension was collected using a syringe and centrifuged at 7000 × g for 10 min before filtering the supernatant through a 0.45-μm cellulose-nitrate filter.

All *in vitro* tests were carried out in duplicate for each soil sample. Soluble Pb concentration in the artificial digestive solution was analyzed using ICP-AES or ICP-MS.

### 2.4. Sequential extraction of soil Pb

Each soil sample (1 g) was weighed into a 40-mL polyethylene centrifuge tube, and the sequential extraction proposed by Tessier et al. [39] was conducted at room temperature as follows:

- **Fraction 1** (water-soluble): soil sample was extracted for 2 h with 15 mL of deionized water.
- **Fraction 2** (exchangeable): the residue from fraction 1 was extracted for 1 h with 8 mL of 1 M MgCl<sub>2</sub> (pH 7.0).
- **Fraction 3** (carbonate-bound): the residue from fraction 2 was extracted for 5 h with 8 mL of 1 M NaOAc adjusted to pH 5.0 with acetic acid (HOAc).
- **Fraction 4** (Fe/Mn-oxide-bound): the residue from fraction 3 was extracted with 20 mL of 0.04 M NH<sub>2</sub>OH·HCl in 25% (v/v) HOAc at 96 °C with occasional agitation for 6 h.
- **Fraction 5** (organic matter-bound): the residue from fraction 4 was extracted with 3 mL of 0.02 M HNO<sub>3</sub> and 5 mL of 30% (v/v) H<sub>2</sub>O<sub>2</sub> (adjusted to pH 2 with HNO<sub>3</sub>). The mixture was heated to 85 °C for 2 h, with occasional agitation. A second 3-mL aliquot of H<sub>2</sub>O<sub>2</sub> (pH 2 with HNO<sub>3</sub>) was added, and the mixture was heated again to 85 °C for 2 h with intermittent agitation. After cooling, 5 mL of 3.2 M NH<sub>4</sub>OAc in 20% (v/v) HNO<sub>3</sub> was added and the sample was diluted to 20 mL and agitated continuously for 30 min.
- **Fraction 6** (residual): the residue from fraction 5 was digested with aqua regia and perchloric acid at 160 °C. A standard reference soil, GSS-1 (National Research Center for Geoanalysis, Beijing, China), was used to verify the acid digestion and instrument performance. The recovery for Pb in GSS-1 was 99.1%.

After each successive extraction, separation was performed by centrifuging at 7000 × g for 10 min. The supernatant was decanted and filtered to remove the plant debris. Before the next extraction,

the residue was washed with deionized water by vigorous manual shaking, and the supernatant was then discarded after 10 min of centrifugation. The Pb concentration in each fraction was determined by ICP-AES or ICP-MS. The overall recovery of Pb using the chemical fractionation procedure, as determined by comparing the sum of Pb determined in all six fractions with a single total Pb determination, was found to be within the range of 91.5–106.8%.

### 2.5. Statistical analysis

The correlation analysis was performed using SPSS 10.0 for Windows between Pb bioaccessibility and single or combined Pb fraction(s) at different incubation times (7 points) for each soil, as well as between Pb bioaccessibility (on day 120) and properties or Pb fraction(s) (on day 120) of different soils (5 points).

## 3. Results and discussion

### 3.1. Temporal variations in Pb fractionation in soils

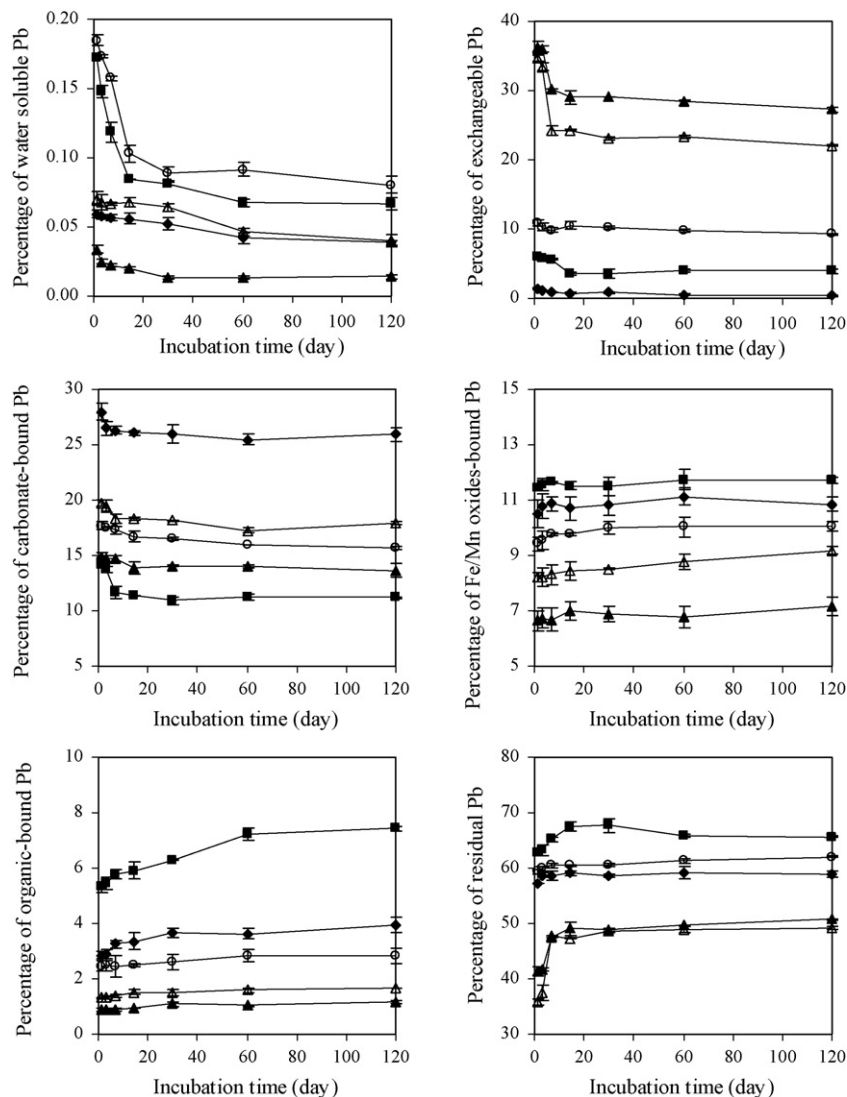
In all studied soils, even at the first sampling (day 1), the addition of Pb resulted in increased Pb concentrations in all six soil fractions (Table 2). However, data clearly show that some redistribution of Pb among the fractions took place during the ensuing incubation (Fig. 1). For soils CC and NJ with their higher pH, the amount of water-soluble Pb (fraction 1) decreased markedly within the first 1–2 weeks following Pb addition. In the case of the other soils (HN, YJ and BJ), only a very small amount of spiked Pb appeared in this fraction, even on day 1 (Table 2). Nevertheless, there was also a slight and slow decrease in the proportion of water-soluble Pb during the first 1–2 months of incubation. Generally, the amount of water-soluble Pb was greater in mildly acidic or alkali soils than in strongly acidic soils after reaching a nearly steady state. Decreases in exchangeable Pb (fraction 2) were also observed during the incubation. The decreases generally occurred more rapidly in the two strongly acidic soils (within 1 week) compared to the mildly acidic or alkali soils (within 2 weeks), except for soil CC. There were differences in the rates at which the proportion of carbonate-bound Pb (fraction 3) generally decreased with time during the first 1–2 weeks of the incubation. The proportions of Fe/Mn-oxide-bound Pb (fraction 4) in the three mildly acidic or alkali soils increased slightly during the first week; in the two strongly acidic soils, however, this fraction continued to increase at very low rates throughout the incubation. Organic-bound Pb (fraction 5) increased sharply in soils BJ and NJ with their relatively high-organic matter content within the first 1–2 months. Only a very slight increase in organic-bound Pb was observed for soil CC with its medium level of organic matter during the first 2 months. For the two strongly acidic soils, with low levels of organic matter, organic-bound Pb increased slightly during the first 2–4 weeks, and then stabilized. Residual Pb (fraction 6) also increased for all studied soils during the incubation. However, the increases were generally completed within the first 1 or 2 weeks of incubation, the proportions of residual Pb remaining stable thereafter.

In summary, there were clear changes in the proportional distribution of Pb in all five studied soils during the first 2 months of incubation with spiked Pb. The proportions of Pb associated with the weakly bound fractions (fractions 1–3) tended to decrease, with corresponding increases in the other three more strongly binding fractions (fractions 4–6). There were differences in the rates at which this redistribution took place, with the changes generally occurring faster in the YJ and HN soils than in the other three soils, probably due to the former's acidic nature.

Despite the differences in the sequential extraction protocol, the results from this study were in general agreement with those of several previous studies [27,29,40]. Here, the magnitude of

**Table 2**  
Pb concentrations (mean  $\pm$  standard deviation) in the fractions of unpolluted and Pb-spiked soils

Soil	Pb concentration in each fraction ( $\text{mg kg}^{-1}$ )					
	Fraction 1	Fraction 2	Fraction 3	Fraction 4	Fraction 5	Fraction 6
<b>CC</b>						
Unpolluted	$0.074 \pm 0.002$	$0.046 \pm 0.002$	$0.47 \pm 0.02$	$2.76 \pm 0.03$	$0.77 \pm 0.01$	$9.19 \pm 0.07$
Pb-spiked (day 1)	$0.87 \pm 0.02$	$51.23 \pm 2.07$	$83.41 \pm 1.50$	$44.39 \pm 0.96$	$11.63 \pm 0.95$	$279.85 \pm 2.73$
Pb-spiked (day 120)	$0.38 \pm 0.02$	$43.78 \pm 1.00$	$74.10 \pm 1.43$	$47.35 \pm 1.44$	$13.26 \pm 1.17$	$292.24 \pm 3.31$
<b>BJ</b>						
Unpolluted	$0.037 \pm 0.003$	$0.045 \pm 0.002$	$1.15 \pm 0.08$	$5.28 \pm 0.03$	$1.30 \pm 0.03$	$19.68 \pm 0.21$
Pb-spiked (day 1)	$0.26 \pm 0.02$	$6.22 \pm 0.24$	$123.16 \pm 1.52$	$46.20 \pm 2.91$	$12.50 \pm 0.80$	$251.85 \pm 4.56$
Pb-spiked (day 120)	$0.19 \pm 0.01$	$1.73 \pm 0.18$	$125.16 \pm 6.56$	$52.34 \pm 2.65$	$18.97 \pm 0.83$	$284.19 \pm 4.45$
<b>NJ</b>						
Unpolluted	$0.072 \pm 0.003$	$0.029 \pm 0.002$	$0.71 \pm 0.02$	$6.45 \pm 0.05$	$1.70 \pm 0.03$	$26.11 \pm 0.40$
Pb-spiked (day 1)	$0.79 \pm 0.01$	$27.38 \pm 1.16$	$66.22 \pm 1.75$	$52.83 \pm 1.00$	$24.48 \pm 0.76$	$289.19 \pm 4.61$
Pb-spiked (day 120)	$0.31 \pm 0.03$	$18.49 \pm 1.21$	$52.47 \pm 0.95$	$54.84 \pm 1.17$	$34.74 \pm 0.73$	$307.37 \pm 4.50$
<b>YJ</b>						
Unpolluted	$0.038 \pm 0.002$	$0.042 \pm 0.002$	$0.59 \pm 0.02$	$4.81 \pm 0.06$	$0.65 \pm 0.02$	$12.83 \pm 0.33$
Pb-spiked (day 1)	$0.15 \pm 0.01$	$166.01 \pm 5.57$	$68.23 \pm 1.26$	$30.50 \pm 1.94$	$4.19 \pm 0.23$	$190.02 \pm 2.36$
Pb-spiked (day 120)	$0.06 \pm 0.01$	$118.92 \pm 2.67$	$59.72 \pm 2.15$	$31.33 \pm 1.80$	$5.15 \pm 0.26$	$221.60 \pm 1.36$
<b>HN</b>						
Unpolluted	$0.024 \pm 0.003$	$0.036 \pm 0.001$	$0.40 \pm 0.01$	$5.29 \pm 0.02$	$0.50 \pm 0.01$	$13.84 \pm 0.16$
Pb-spiked (day 1)	$0.31 \pm 0.01$	$150.64 \pm 2.00$	$85.62 \pm 0.83$	$35.68 \pm 1.74$	$5.71 \pm 0.33$	$155.95 \pm 6.52$
Pb-spiked (day 120)	$0.17 \pm 0.02$	$95.98 \pm 1.40$	$77.91 \pm 2.20$	$39.98 \pm 1.25$	$7.23 \pm 0.19$	$213.88 \pm 2.53$



**Fig. 1.** Variation of Pb fractionation in soils with incubation time (○, (◆), (■) (▲) and (△) represent soils CC, BJ, NJ, YJ and HN, respectively).

the decrease in the exchangeable Pb fraction was much larger in strongly acidic soils than in mildly acidic or alkali soils. Mineral dissolution has been reported to predominate under acidic conditions and to cause a decrease in exchangeable Pb with time due to the dissolution of Fe oxides [40]. In contrast, a constant level of exchangeable Pb throughout the entire incubation period was reported by Fendorf et al. [28], a difference which may be due mainly to differences in the method of Pb addition and subsequent soil incubation. Fendorf et al. [28] added Pb in a 1:10 (w:v) soil-to-solution ratio and incubated the soil slurry on an orbital shaker for 10 h, providing ideal conditions for Pb partitioning into the various soil components via the solution phase and the achievement of a nearly steady state within a relatively short time; thereafter, they maintained an approximately 33% moisture content for the duration of the experiment with no wetting–drying cycle. There were also slight declines in the carbonate fraction of Pb with time in our study, in agreement with the decreases in the amount of Pb retained in the carbonate fraction with time at pH values of 6–8 observed by Lim et al. [40]. Nevertheless, inconsistent changes in Pb in the carbonate fraction in three soils from China were reported by Lu et al. [29]. The magnitude of the decrease in exchangeable Pb in the two strongly acidic soils (YJ and HN) was close to that of the increase in residual Pb during the first week, indicating that transformation of exchangeable Pb to the least available residual Pb could be a dominant process. In contrast, for mildly acidic or alkali soils (CC, BJ and NJ), the loosely bound fractions, i.e. the water-soluble, exchangeable and carbonate fractions, transformed to more strongly bound fractions, particularly the organic and residual fractions, during the first week. It has been reported that water-soluble, exchangeable, and EDTA-extractable (assumed to be specifically sorbed) Pb in a Mollisol transforms to the easily reducible Mn-oxide- and Fe-oxide-bound forms within 1 month, and the slow process of transformation was attributed mainly to the diffusion of the surface species into micropores and their entrapment in microporous solids [27].

Of all of the soil's properties, pH and clay minerals appear, in this study, to be the most important factors affecting Pb redistribution in strongly acidic soils following Pb addition. Low soil pH seems to favor the quick redistribution of Pb from the exchangeable fraction to the residual fraction. The dominant clay mineral, kaolinite, which is a 1:1 clay mineral characterized by relatively low CEC and affinity for heavy metals [41], together with soil pH, were probably responsible for the relatively low level of Pb in the residual fraction of the two strongly acidic soils in the nearly steady state. In contrast, for the three mildly acidic or alkali soils dominated by 2:1 clay mineral(s) having relatively high CEC and affinity for heavy metals [41], more soil factors, including soil pH, carbonate, organic matter and clay minerals, affected Pb redistribution with time.

There were some clear and major differences between the distributions of indigenous and spiked Pb in the nearly steady state, but there were also differences between some of the individual soils (Table 2). In the case of indigenous Pb, both concentrations and proportions of the water-soluble, exchangeable, carbonate and organic-bound fractions were very low for all studied soils, with the bulk of the Pb being distributed between the Fe/Mn-oxide and residual fractions. In particular, the proportion of the dominant residual fraction of indigenous Pb was over 67.67% for all studied soils with a mean value of 70.35%. In contrast, the main body of the spiked Pb appeared in the exchangeable, carbonate, Fe/Mn-oxide and residual fractions, as indicated in Table 2.

In the nearly steady state, the proportion of exchangeable Pb was much higher in strongly acidic soils (22.06–27.23%) than in mildly acidic or alkali soils (0.36–9.29%). The highest proportion of carbonate-bound Pb was found in soil BJ with the highest carbonate content relative to the other four soils. The Fe/Mn-oxide-bound

Pb fraction in mildly acidic or alkali soils (10.05–11.71%) was higher than that in strongly acidic soils (7.17–9.19%) on day 120, possibly because the lower pH of the two latter soils make Fe/Mn oxides more positively charged, reducing their ability to bind Pb, despite their higher contents in such soils. Residual Pb levels in mildly acidic or alkali soils were obviously higher than those in the strongly acidic soils in the nearly steady state, possibly as a result of the higher soil pH and dominance of 2:1 clay minerals in soils CC, BJ and NJ.

There are a number of previous reports characterizing Pb fractionation in unpolluted and polluted soils using various sequential extraction procedures [27,29]. In an artificially polluted Mollisol, EDTA-extractable (57%), residual (26%) and easily reducible Mn-bound (10%) fractions were reported to be the dominant forms of added Pb [27]. Nevertheless, Lu et al. [29] reported that added Pb enters mainly the exchangeable, Fe/Mn-oxide and organic fractions of three soils from China. In sludge-amended soils, Pb was found to be mainly associated with metal oxides and other soil minerals rather than organic matter, which agrees with other studies on the behavior of Pb in soils [42]. In two contrasting shooting-range soils (TRR and MPR), residual Pb was predominant (20.7%) in the TRR soil primarily as a result of the formation of hydroxypyromorphite due to the presence of adequate P in the soil; however, Pb in the MPR soil was primarily associated with the carbonate fraction (59.8%), followed by organic > Fe/Mn-oxide > residual > water-soluble/exchangeable fractions [43]. Ettler et al. [44] reported that Pb originating from metallurgical processing is mainly bound to the exchangeable and organic fractions in forest soil; in contrast, it was predominantly associated with the Fe/Mn-oxide fraction in tilled soil. Generally, the great variations in reported soil Pb fractionation within each study or among different studies may be attributed not only to the differences in the origin (indigenous or pollution) of the Pb, the pollution source, the soil properties (soil pH, organic matter and clay mineralogy) and the phosphate supply or application, but also to differences in the sequential extraction procedures used. Therefore, our study offers insight into the partitioning and redistribution of spiked free Pb ions among various soil components in different soil types.

The distribution of added Pb into the different solid phases may be assumed to be a multistep process involving initial rapid retention followed by secondary slow retention. The rapid retention is initiated by instantaneous adsorption to soil surfaces via the formation of outer-sphere (electrostatic or physical) complexes [28] driven by the concentration gradient from the solution phase to the surface of soil minerals and negatively charged organic matter. Initial adsorbed Pb is an easily exchangeable fraction, which is retained at a high level at the beginning of the incubation. Therefore, this rapid absorption may have been completed within the 1 day before the monitoring in this study was initiated. Following the rapid absorption, a secondary (slower) shift of Pb from outer to inner spheres (micropores) [28], possibly involving Fe/Mn oxides, organic matter and the edges of the soil minerals (residual fraction), may occur. Further ageing may lead to more extensive changes, including nucleation and the development of a surface precipitate [45], and occlusion by organic or inorganic materials [46]. Apart from the soil adsorption process, coprecipitation (solid solution) with amorphous Fe oxides and precipitation as pyromorphite upon high Pb loading and the application of adequate phosphoric acid or phosphate could also effectively contribute to removing Pb from the solution [20,21,47]. The rate of slow retention declines with time to equilibrium among the different Pb fractions via the soil solution. Therefore, the time needed to approach an apparent equilibrium may be greatly affected by soil pH, clay minerals, Pb-loading rate, organic matter and soluble P content.

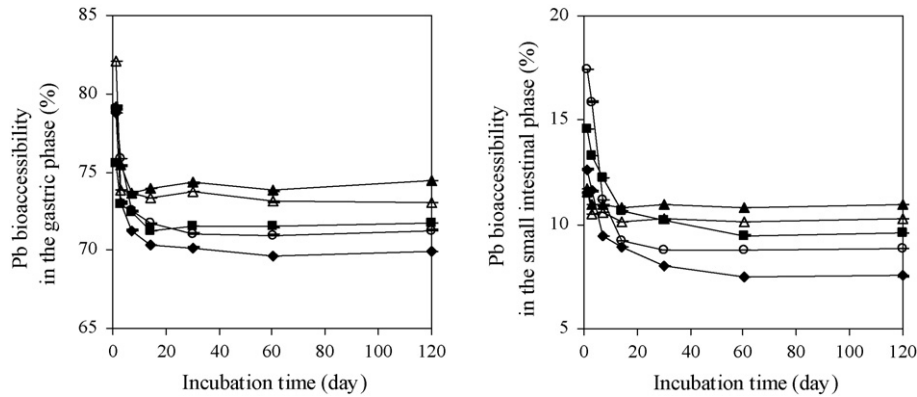


Fig. 2. Variation of Pb bioaccessibility in soils with incubation time for both the gastric and small intestinal phases ((○), (◆), (■), (▲) and (△) represent soils CC, BJ, NJ, YJ and HN, respectively).

### 3.2. Temporal variations in Pb bioaccessibility in soils

For mildly acidic or alkali soils (CC, BJ and NJ), Pb bioaccessibility decreased exponentially to a nearly steady level in the gastric and small intestinal phases during the first 2–4 weeks and 1–2 months of incubation, respectively; however, it took only 1–2 weeks for the strongly acidic soils (YJ and HN) to reach a nearly steady level of Pb bioaccessibility in both phases (Fig. 2). In contrast, the decrease in bioaccessibility occurred faster in strongly acidic soils compared with mildly acidic or alkali soils. An exponential decrease in Pb bioaccessibility of soil in the gastric phase with time within the first 2 weeks was also reported by Fendhorf et al. [28].

Pb bioaccessibility was lower than 100% for all five soils in both the gastric and small intestinal phases. In the nearly steady state, Pb bioaccessibility in both phases in the strongly acidic soils (YJ and HN) was also higher than that in the other soils, reflecting the important role of soil pH in controlling Pb bioaccessibility (Fig. 2). Bioaccessible Pb level in soil BJ, characterized by the highest soil pH at 7.43, appeared to be the lowest at the end of the incubation (day 120). Pb bioaccessibility in soils in both phases followed the same order: YJ > HN > NJ > CC > BJ. In agreement with the observations by other researchers [10,19], for both polluted and unpolluted soils in this study, Pb bioaccessibility in the small intestinal phase was markedly lower than that in the gastric phase, possibly resulting from the extensive Pb adsorption and precipitation at relatively high solution pH in the small intestinal phase. This suggests that soil Pb will show higher bioaccessibility in the early stages of pollution, especially in strongly acidic soils that are usually low in organic matter but high in Fe/Mn-oxide contents.

There have been a number of studies estimating Pb bioaccessibility in soils using various *in vitro* tests. Ruby et al. [10] reported that dissolution of Pb in the acidic gastric phase is strongly pH-dependent, with the extent of dissolution decreasing by 65% as the gastric pH increases from 1.3 to 2.5. On entering the small intestinal phase, Pb bioaccessibility decreased by 74% [10]. The addition of phosphorous fertilizers and phosphoric acid was proven to be effective in reducing the bioaccessible and leachable Pb as a result of its transformation in contaminated soils to (chloro)pyromorphite [18–21]. Moreover, Pb bioaccessibility in 15 soils from Germany was increased from 3–20% to 11–56% by the addition of powdered milk, possibly via the formation of soluble Pb complexes with various milk constituents [17].

Pb bioaccessibility in unpolluted soils in both phases followed this order (Pb bioaccessibility in the gastric phase/Pb bioaccessibility in the small intestinal phase): YJ (63.48/9.07%) > HN (60.17/8.48%) > NJ (53.09/5.91%) > CC (51.43/4.22%) > BJ (49.62/3.36%). Pb bioaccessibility in unpolluted soils in both phases was much lower

than that in artificially polluted soils in the nearly steady state. These observations suggest that the artificially added Pb is much more labile and bioaccessible than indigenous Pb and thus poses a greater health risk to humans.

### 3.3. The relationship between Pb bioaccessibility and fractionation in soils

Correlations between Pb bioaccessibility and single or combined soil Pb fraction(s) at different incubation times were determined for each soil, in an attempt to reveal the possible sources of Pb available for the small intestinal absorption (Table 3). Bioaccessible Pb was positively related to Pb in either fractions 1, 2 or 3 individually (mostly at significant levels for all of the experimental soils in either digestive phase) or in the sum of fractions 1–3 (always at significant levels for mildly acidic or alkali soils in either phase, and at significant levels for strongly acidic soils in either the gastric or small intestinal phase). Pb bioaccessibility was negatively related to fraction 6 at significant levels ( $P < 0.05$  or  $P < 0.01$ ) for mildly acidic or alkali soils but not always significantly for strongly acidic soils in both phases. Nevertheless, it should be noted that neither the proportion of individual fractions 1–3 nor the sum of these three fractions was consistent with bioaccessible Pb level in the gastric phase with respect to its magnitude and its decline with time.

An additional regression analysis was performed between bioaccessible Pb and sequentially extracted Pb fraction(s) or selected soil properties across all the studied soils (5 points, day 120), for the data obtained at the final sampling after 4 months of incubation (Table 4). The results showed that Pb bioaccessibility is positively correlated with Pb in fraction 2, in both the gastric ( $P < 0.05$ ) and the small intestinal phase (not at a significant level), across all five soils. Both soil pH and organic-carbon content was negatively correlated at significant levels to bioaccessible Pb in both digestive phases, and the content of  $\text{CaCO}_3$  was negatively correlated ( $P < 0.05$ ) to bioaccessible Pb level in the small intestinal phase but not in the gastric phase. Moreover, the clay mineralogy also appeared to affect Pb bioaccessibility, as reflected by the lower levels of bioaccessible Pb observed in mildly acidic or alkali soils dominated by 2:1 clay minerals (e.g. illite, smectite and vermiculite) compared with the strongly acidic soils dominated by 1:1 clay mineral (e.g. kaolinite) or Fe oxides (e.g. gibbsite and hematite) at the end of the incubation. However, no significant relationship between Pb bioaccessibility and CEC was observed in this study (Table 4).

We found that throughout the entire incubation time, the sum of Pb fractions 1–5 was lower than the bioaccessible Pb level in the gastric phase but markedly higher than that in the small intestinal phase. It has been reported that soil Pb bound

**Table 3**  
Correlation between single or combined Pb fraction(s) and Pb bioaccessibility in the gastric and small intestinal phases for each Pb-spiked soil throughout the incubation period

Pb bioaccessibility	CC		BJ		NJ		YJ		HN	
	Small intestinal		Gastric		Small intestinal		Gastric		Small intestinal	
	Gastric	Small intestinal	Gastric	Small intestinal	Gastric	Small intestinal	Gastric	Small intestinal	Gastric	Small intestinal
Fraction 1	0.884* (0.008) <sup>a,b</sup>	0.930** (0.002)	0.604 (0.151)	0.781* (0.038)	0.901** (0.006)	0.997** (0.000)	0.817* (0.025)	0.786* (0.036)	0.436 (0.329)	0.466 (0.292)
Fraction 2	0.700 (0.080)	0.649 (0.115)	0.875** (0.01)	0.935** (0.002)	0.806* (0.029)	0.908** (0.005)	0.773* (0.042)	0.660 (0.107)	0.736 (0.059)	0.781* (0.038)
Fraction 3	0.811* (0.027)	0.854* (0.015)	0.978** (0.000)	0.888** (0.008)	0.897** (0.006)	0.929** (0.002)	0.594 (0.159)	0.589 (0.164)	0.718 (0.069)	0.791* (0.034)
Fraction 1+2+3	0.823* (0.023)	0.829* (0.021)	0.973** (0.000)	0.929** (0.003)	0.890** (0.007)	0.957** (0.001)	0.764* (0.046)	0.662 (0.105)	0.741 (0.057)	0.790* (0.034)
Fraction 4	-0.937** (0.002)	-0.945** (0.001)	-0.768* (0.044)	-0.743 (0.056)	-0.500 (0.253)	-0.611 (0.145)	-0.416 (0.353)	-0.389 (0.387)	-0.463 (0.296)	-0.515 (0.237)
Fraction 5	-0.591 (0.162)	-0.614 (0.142)	-0.795* (0.033)	-0.956** (0.001)	-0.624 (0.134)	-0.881* (0.009)	-0.302 (0.510)	-0.249 (0.591)	-0.610 (0.146)	-0.713 (0.072)
Fraction 6	-0.794* (0.033)	-0.797* (0.032)	-0.940** (0.002)	-0.770* (0.043)	-0.844* (0.017)	-0.812* (0.026)	-0.777* (0.040)	-0.672 (0.098)	-0.744 (0.055)	-0.791* (0.034)

<sup>a</sup> The significance *P* values are shown in parentheses.

<sup>b</sup> \* and \*\* represent 5 and 1% levels of significance (two-tailed), respectively.

**Table 4**

Correlation between Pb bioaccessibility and sequentially extracted Pb fraction or selected soil properties in the gastric and small intestinal phases after 4 months of incubation

	Pb bioaccessibility	
	Gastric	Small intestinal
Fraction 1	-0.560 (0.326)	-0.406 (0.497)
Fraction 2	0.940* (0.017) <sup>a,b</sup>	0.877 (0.051)
Fraction 3	-0.602 (0.283)	-0.725 (0.166)
Fraction 4	-0.823 (0.087)	-0.686 (0.201)
Fraction 5	-0.515 (0.374)	-0.366 (0.545)
Fraction 6	-0.672 (0.214)	-0.558 (0.328)
Soil pH	-0.953* (0.012)	-0.959** (0.010)
OC	-0.889* (0.044)	-0.887* (0.045)
CEC	-0.562 (0.324)	-0.461 (0.435)
Free Fe oxide	0.635 (0.250)	0.641 (0.244)
Free Mn oxide	-0.007 (0.991)	0.192 (0.756)
CaCO <sub>3</sub>	-0.786 (0.115)	-0.882* (0.048)

<sup>a</sup> The significance *P* values are shown in parentheses.

<sup>b</sup> \* and \*\* represent 5 and 1% levels of significance (two-tailed), respectively.

to carbonate and weak organic complexes, Mn oxides and poorly crystalline Fe-oxihydroxides are significantly depleted by *in vitro* gastrointestinal-extraction treatment [17]. Therefore, it is likely that the gastric solution, which has a lower pH (1.5) than the extractants of fractions 1–5 but differs in ionic composition, releases Pb from soils, via its desorption and/or dissolution, from the carbonate, Fe/Mn-oxide, organic matter and even clay–mineral phases, indicating that bioaccessible Pb in the gastric phase may come from all soil fractions, even the residual one. Despite the positive relationship between bioaccessible and exchangeable Pb, the difference in Pb bioaccessibility in the gastric phase among the five studied soils at the end of incubation period was likely due to the difference in binding affinity of the residual fraction for Pb under acidic conditions. In contrast, the neutral pH (7.0) of the small intestinal phase, which is identical to the pH of the extractant of fraction 2 but with higher ionic strength, favors the adsorption of Pb, whereas Pb dissolution from soil minerals might be very low. To identify the sources of bioaccessible Pb in soils, in addition to correlation analyses between Pb bioaccessibility and single or combined Pb fraction(s) in soils at different incubation times or across different soils at the end of incubation, an additional comparison, of the magnitude of Pb bioaccessibility and single or combined sequentially extracted soil Pb fraction(s) was performed. These results showed that, in addition to the water-soluble and exchangeable fractions, the carbonate fraction in mildly acidic or alkali soils appears to be another important source of bioaccessible Pb in the small intestinal phase; however, in strongly acidic soils, bioaccessible Pb in the small intestinal phase is likely to derive principally from water-soluble Pb and a small proportion of exchangeable Pb.

#### 4. Conclusions

The bioaccessibility of Pb added to relatively unpolluted soils decreased exponentially to a nearly steady level in mildly acidic or alkali soils (CC, BJ and NJ), this occurred within the first 2–4 weeks for the gastric phase and 1–2 months of incubation for the small intestinal phase. However, it took only 1–2 weeks for the strongly acidic soils (YJ and HN) to reach a nearly steady level of Pb bioaccessibility in both phases. Soil pH, clay mineralogy and organic-carbon content were probably the principal factors affecting Pb bioaccessibility once this steady level was attained. However, the content of CaCO<sub>3</sub> was found to be negatively correlated ( $P < 0.05$ ) to bioaccessible Pb levels in the small intestinal phase. At the end of the incubation, the main body of spiked Pb was found in the carbonate, Fe/Mn-oxide and residual fractions for mildly acidic or alkali soils,

whereas for strongly acidic soils, it was found in the exchangeable, carbonate and residual fractions. Soil pH along with clay mineralogy appeared to be the principal factors controlling Pb redistribution in strongly acidic soils following Pb addition. In contrast, for mildly acidic or alkali soils, more soil factors, including soil pH, carbonate, organic matter and clay minerals, affected Pb redistribution with time. Bioaccessible Pb in the gastric phase appeared to derive from all of the fractions in the soils, even the residual fraction, possibly due to the strong Pb desorption and/or dissolution from carbonate, Fe/Mn oxides, organic matter and even clay minerals under the highly acidic conditions (pH 1.5) of the simulated gastric solution. In addition to the water-soluble and exchangeable fractions, the carbonate fraction of mildly acidic or alkali soils appeared to be a third main pool of bioaccessible Pb in the small intestinal phase; however, bioaccessible Pb was likely to derive principally from Pb in the water-soluble and exchangeable fractions of strongly acidic soils. Even though China's major soil types were represented in the five soil samples used in this study, future studies using soils from a larger number of sites with different contamination histories are recommended.

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